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Minireview

Role of thylakoid protein kinases in photosynthetic acclimation

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Abstract Photosynthetic organisms are able to adjust to changes in light quality through state transition, a process which leads to a balancing of the light excitation energy between the antennae systems of photosystem II and photosystem I. A genetic approach has been used in Chlamydomonas with the aim of elucidating the signaling chain involved in state transitions. This has led to the identification of a small family of Ser–Thr protein kinases associated with the thylakoid membrane and conserved in algae and land plants. These kinases appear to be involved both in short and long term adaptations to changes in the light environment.

Keywords: Light acclimation; State transitions; Thylakoid membrane; Kinase; Light-harvesting system; ATP

1. Introduction

The primary reactions of photosynthesis in algae and plants occur in the chloroplast thylakoid membranes and are catalyzed by the multi protein-pigment complexes photosystem II (PSII), the cytochrome b_6f complex and photosystem I (PSI) (Fig. 1). Light excitation energy is collected by the antennae of PSII and PSI and channeled to their reaction centers. At the level of PSII this leads to the photooxidation of the P680 chlorophyll dimer and the subsequent splitting of water with the release of molecular oxygen, protons and electrons which are fed into the photosynthetic electron transport chain through PSII, the plastoquinone pool, the cytochrome b_6f complex, plastocyanin and, ultimately, these electrons are used to re-reduce the photooxidized PSI reaction center which acts as a light-driven plastocyanin–ferredoxin oxido-reductase.

In order to survive and grow optimally, photosynthetic organisms need to adapt constantly to changing light conditions. Both short term and long term adaptations occur. Short term adaptations take place in the second to minute range and include feedback de-excitation, a process which occurs when the incident light energy exceeds the capacity of the photosynthetic machinery. The excess energy is released as heat through changes in the thylakoid membrane which involve the PsbS protein and activation of the xanthophyll cycle (reviewed in [1]). Under changing low light conditions photosynthetic organisms optimize their photosynthetic yield through a process called state transition which leads to balancing of the light excitation energy between PSII and PSI whenever one of the two photosystems is preferentially sensitized by the incident light. This response also includes reversible phosphorylation of thylakoid membrane proteins. In contrast, long term adaptations occur over several hours or days and involve changes in the stoichiometry of the photosystems and in the amounts of their antennae [2].

Considerable progress has been achieved in recent years in elucidating some of the molecular mechanisms which underlie the dynamics of these acclimation processes through the combined use of physiological, molecular genetic, biochemical and biophysical approaches. Here I review recent advances in understanding state transitions with a comparative analysis between *Chlamydomonas reinhardtii* and the land plant *Arabidopsis thaliana*. Comparison of this acclimation process in a green motile unicellular alga and a sessile land plant has provided interesting insights into evolutionary aspects of this response. Several excellent earlier reviews have appeared on state transitions [3,4].

2. The process of state transition

PSII and PSI operate in series within the photosynthetic electron transport chain. Their antenna systems preferentially absorb 650 and 700 nm light, respectively. Because of these differences in light absorption properties, changes in light conditions can lead to unequal excitation of the two photosystems and thus to a decreased photosynthetic yield. Plants and algae are able to balance the absorbed light energy between the two photosystems through state transitions, a process which was discovered nearly 40 years ago [5,6]. When PSII is overstimulated relative to PSI, the redox state of the PQ pool is changed to a more reduced state. These conditions favor docking of plastoquinol (PQH_2) to the Qo site of the cytochrome b_6f complex, which leads to the activation of a thylakoid protein kinase which phosphorylates the LHCII complex [7,8]. A fraction of this complex, the mobile part of LHCII, moves subsequently from PSII to PSI thus balancing the light excitation energy between the two photosystems. This state in which the mobile part of LHCII is associated with PSI is called state 2 (Fig. 1). When plants or algae are illuminated by light that is

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Abbreviations: PSII, photosystem II; PSI, photosystem I; LHC, light-harvesting complex; PQ, plastoquinone; PQH_2, plastoquinol; DCMU, 3-(3,4-dichlorophenyl)1,1-dimethylurea

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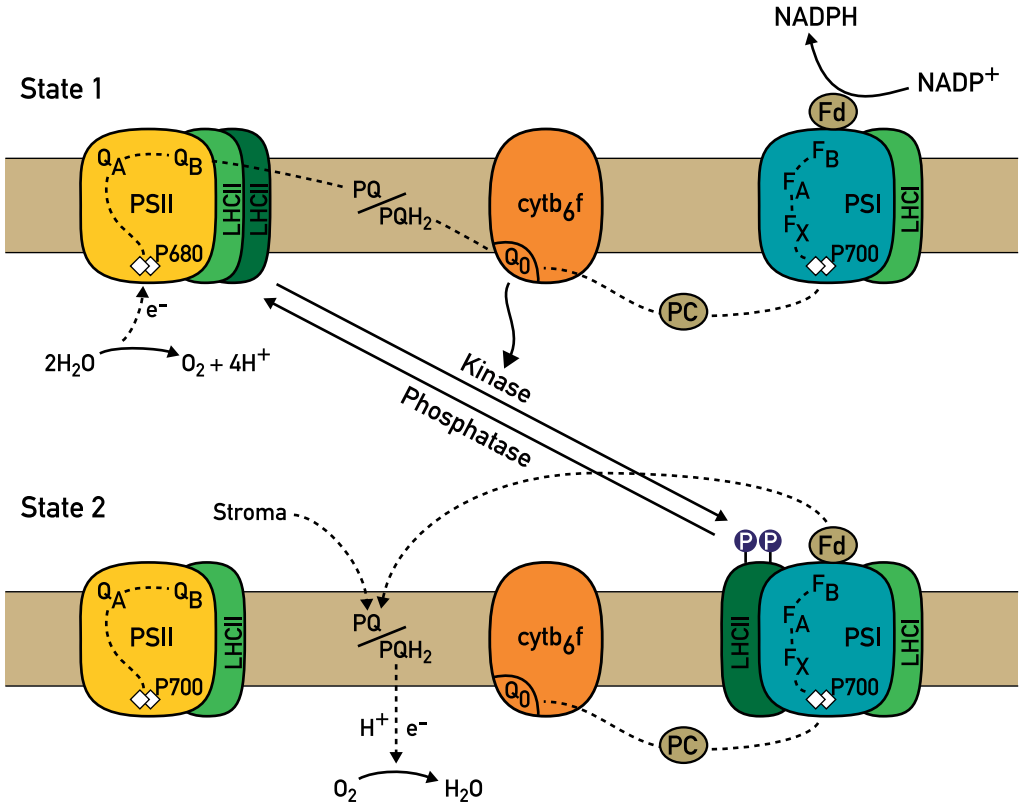

Fig. 1. Scheme of photosynthetic complexes in the thylakoid membrane and state transitions. State transitions are determined to a large extent by the redox state of the PQ pool. Preferential excitation of PSI leads to the oxidation of the PQ pool and to state 1. In state 1 the mobile light-harvesting antenna is bound to PSII and the photosynthetic electron transport chain acts mostly in a linear mode generating NADPH and ATP. Preferential excitation of PSII relative to PSI leads to a reduced state of the plastoquinone pool and thus to the docking of plastoquinol to the Qo site of the cytochrome b_6f complex. This activates the LHCII kinase and leads to the displacement of phosphorylated LHCII from PSII to PSI (state 2). In state 2 in *C. reinhardtii*, PSII is disconnected from the rest of the electron transport chain which acts mostly in a cyclic mode. State 2 can also be induced in the dark through the chloro-respiratory electron transport chain under anaerobic conditions which prevent the oxidation of the plastoquinone pool or in response to reduced levels of ATP. Electron transfer chains are represented as dotted lines. P680, PSII reaction center chlorophyll dimer, Q_A, Q_B, primary and secondary electron acceptors of PSII.; P700, PSI reaction center chlorophyll dimer, F_X, F_A, F_B, 4Fe–4S centers acting as electron acceptors within PSI; PQ, plastoquinone pool; PC, plastocyanin; Fd, ferredoxin.

absorbed preferentially by PSI, the PQ pool becomes oxidized. This leads to the inactivation of the LHCII kinase, the dephosphorylation of the LHCII associated with PSI through some unknown phosphatase and the return of LHCII to PSII. This state is called state 1 (reviewed in [3,4]).

Thylakoid membranes consist of stromal lamellae and appressed membranes which constitute the grana. It is noteworthy that PSII is localized within the grana regions whereas PSI is mostly associated with the stromal lamellae [9]. Thus state transitions could involve a redistribution of the light-harvesting antennae over several hundreds of nanometers on the thylakoid membranes. However, an alternative view places functional PSI near the grana ends close to PSII making long range diffusion of LHCII unnecessary [10,11].

In *C. reinhardtii* state transitions are not only involved in the rebalancing of the excitation energy between the photosystems following changes in light conditions. The major role of state transitions appears to be the restoration of ATP levels when the intracellular level of ATP is low [12]. This induces transition to state 2. Interestingly transition from states 1 to 2 involves a reorganization of the photosynthetic electron transport chain in this alga. PSII is largely disconnected from the electron transport chain which switches into a cyclic mode [13]. Thus state 1 is associated with linear electron transport and acts as generator of reducing power and ATP whereas state 2 is associated with a cyclic mode which produces exclusively ATP. A role in ATP adjustment may explain why as much as 80% of LHCII is mobile during state transitions in *C. reinhardtii* whereas the corresponding number is only 15–20% in land plants [14].

3. Mobility of LHCII during state transitions

During a states 1–2 transition, a thylakoid protein kinase phosphorylates LHCII. This complex includes LHCII proteins which are encoded in *C. reinhardtii* by a small gene family consisting of nine members with high sequence similarity [15,16]. Each of these proteins binds eight chlorophyll *a*, six chlorophyll *b* and several carotenoid and xanthophyll molecules. The determination of the structure of LHCII has revealed that the protein contains three *trans*-membrane helices and a short amphipathic helix exposed to the lumenal side of the thylakoid membrane [17,18]. The major LHCII proteins form trimers. Moreover in land plants three monomeric LHCII proteins CP29, CP26 and CP24 encoded by the Lhcb4, Lhcb5 and Lhcb6 genes, respectively, provide a connection between the LHCII trimers and the reaction center of PSII.

In *C. reinhardtii* orthologues of CP29 and CP26 exist but not of CP24 [15].

Several thylakoid membrane proteins from the PSII complex and LHCII undergo light- and redox-dependent phosphorylation [19,20]. However because of the numerous LHCII proteins with similar sizes and sequences, it has been difficult to determine unambiguously which of the LHCII proteins are phosphorylated and which residues within these proteins are phosphorylated. Although several thylakoid phosphoproteins were identified by classical means including ^{32}P-phosphate labeling, immunoblotting with anti-P-Thr antibodies, and by observing shifts in electrophoretic mobility, mass-spectrometry has provided a more comprehensive picture of thylakoid protein phosphorylation [21]. A powerful method consists in treating isolated thylakoid membranes with trypsin to release the N-terminal phosphorylated regions of the LHCII proteins which are exposed to the stroma. These are enriched by metal affinity chromatography and subjected to matrix-assisted laser desorption/ionization and electrospray ionization mass-spectrometry. Analysis of thylakoid membranes from *C. reinhardtii* cells in states 1 and 2 or exposed to high light identified a total of 19 in vivo phosphorylation sites corresponding to 15 polypeptides [21]. Besides revealing phosphorylation of the PSII core proteins D1 and CP43 at their N-terminal Thr, this study also showed that the LHCII proteins Lhcbm4, Lhcbm6, Lhcbm9 and Lhcbm11 are all phosphorylated at Thr3. A particularly interesting finding is that CP29 undergoes sequential multiple phosphorylations. Two sites are phosphorylated under state 1 conditions, two additional sites are phosphorylated under state 2 conditions and three additional sites are phosphorylated under high light. The principal changes in phosphorylation which occur during state transitions are clustered at the interface between the PSII core and the associated light-harvesting antenna proteins which include LHCII, CP26 and CP29. In this respect the differential phosphoryl- ation of CP29 appears to play a key role in state transitions, at least in *C. reinhardtii* (Fig. 2).

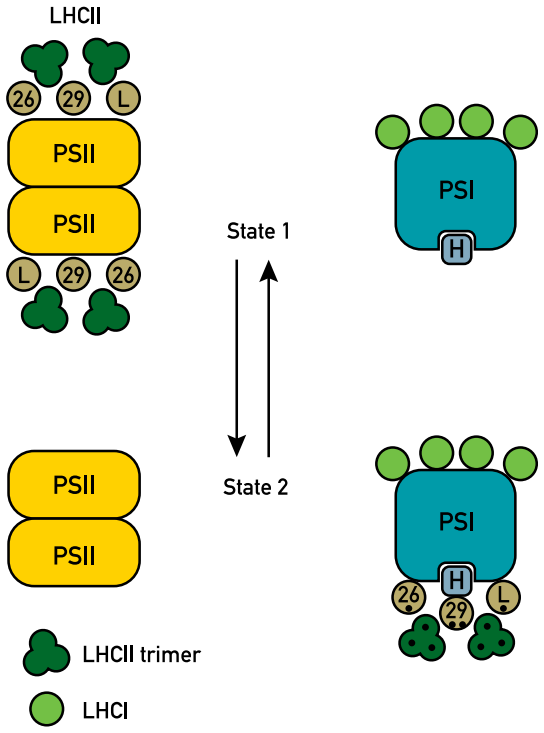

Fig. 2. Displacement of mobile LHCII from PSII to PSI during a transition from states 1 to 2 in *C. reinhardtii*. The monomeric LHCII proteins CP26 (26), CP29 (29) and Lhcbm5 (L) act as connectors between the PSII core dimer and the trimeric LHCII [24]. It is likely that phosphorylation of the monomeric LHCII proteins plays a key role in inducing transition from states 1 to 2. Several phosphorylation sites are located at the interface between the PSII core and LHCII. Phosphorylation sites are indicated by dots. Docking of LHCII occurs on the PsaH side of PSI whereas the LHCI belt is on the other side of PSI [21,22].

Upon transition from states 1 to 2, the mobile LHCII is displaced from PSII in the grana to PSI in the stromal lamellae. A fraction of the cytochrome b_6f complexes also migrates from the grana to the stromal lamellae under these conditions [22]. While the evidence for this process was mostly based on spectroscopic data and immuno-electron microscopy, it is only recently that biochemical evidence for an association of LHCII polypeptides to PSI in state 2 was provided through the isolation of a PSI–LHCI complex from Arabidopsis plants [23]. Crosslinking studies revealed that the docking site of LHCII is formed by the PsaH, PsaI and PsaO subunits of PSI. These results agree with the finding that Arabidopsis lines in which PsaH expression has been suppressed are deficient in state transitions [24]. According to the recently determined 4.4 A crystal structure of the PSI–LHCI complex from land plants, PsaH is located in an exposed hydrophobic region of PSI and PsaH binds a chlorophyll molecule which could mediate energy transfer from LHCII to the PSI core [25]. Additional support for an association of LHCII with PSI is provided by recent studies with *C. reinhardtii* [26,27]. A PSI–LHCI supercomplex was isolated from cells in state 2 which is distinctly larger than the corresponding complex from cells in state 1. In addition to the LHCI polypeptides this supercomplex from cells in state 2 contains the three LHCII proteins CP29, CP26 and Lhcbm5 [26,27]. Lhcbm5 is a LHCII type II protein which accumulates to similar levels than CP29 and CP26 but at a much lower level than the other major LHCII proteins [27]. Both CP29 and Lhcbm5 are phosphorylated when they are associated with PSI. Thus Lhcbm5 could occupy a similar position as CP24 in land plants and together with CP29 and CP26 it would act as a linker between the PSII core dimer and the trimeric LHCII in state 1 whereas in state 2 these three proteins would migrate to PSI on the PsaH side and allow for the docking of LHCII trimers to the PSI complex. Interestingly according to the PSI–LHCI crystal structure, four Lhca subunits form a light-harvesting belt on the opposite side of PsaH [25] (Fig. 2). Thus CP29, CP26 and Lhcbm5 which were thought to belong exclusively to the PSII complex appear to shuttle between PSII and PSI during state transitions and provide docking sites for the trimeric LHCII proteins [27]. The quadruple phosphorylation of CP29 in state 2 could lead to the dissociation of CP29-LHCII from PSII to PSI whereas under high light the phosphorylation of three additional residues of CP29 could induce the dissociation of LHCII from the photosystems and allow for thermal energy dissipation within the LHCII trimers [28,29]. It therefore appears that state transitions in Chlamydomonas are regulated by reversible protein phosphorylations at the interface between the PSII core and LHCII including in particular the phosphorylation of the linker protein CP29 and probably also Lhcbm5.

Mutants of *C. reinhardtii* lacking PSI are blocked in state 1. Although these mutants are able to phosphorylate LHCII under state 2 conditions, LHCII remains functionally connected to PSII [14]. Similarly, Arabidopsis lines lacking PsaH are also blocked in state 1 [24]. They still phosphorylate LHCII under state 2 conditions and LHCII remains connected to PSII. These results indicate that the association of the phosphorylated LHCII with PSI and PSII is a competitive process which is determined by the relative binding affinities of phosphorylated LHCII to the two photosystems [4].

4. An elusive LHCII kinase

With numerous thylakoid proteins phosphorylated under different environmental conditions, the question arises how many different kinases are involved. Following the discovery of LHCII kinase activity in 1977 by Bennett [19], a long and intensive biochemical search for this kinase was undertaken. Although several thylakoid protein kinase activities could be identified, attempts to purify the LHCII kinase by biochemical means were unsuccessful [30,31]. Therefore, new approaches were chosen. Because the N-terminal region of the light-harvesting proteins contains the residues that are phosphorylated during states 1–2 transition, Kohorn and colleagues screened for proteins that interact with this region [32]. In this way they identified a small family of three kinases, called TAK kinases (thylakoid associated kinases) on the thylakoid membranes of Arabidopsis. The TAK kinases contain a repeated Gly–Ser motif which is also present in human transforming growth factor β1 receptor. Although they phosphorylate LHCII in vitro it is difficult to assess their specificity. Analysis of TAK antisense lines with decreased levels of TAK revealed increased sensitivity to high light, a decrease in LHCII phosphorylation and a partial deficiency in state transitions [33]. It is not yet clear whether TAK kinases are unique to land plants or whether they are also present in algae, and their exact role has not yet been elucidated.

Because of the failure to identify the LHCII kinase through biochemical means, a genetic approach was initiated in *C. reinhardtii* with the ultimate aim of identifying not only the LHCII kinase, but also other factors of the signal transduction chain of state transitions [34,35]. *C. reinhardtii* is ideally suited for this type of screen because large fluorescence changes occur during a transition from states 1 to 2. This is due to the fact that most of the LHCII antenna is displaced from PSII to PSI during this process and because the room temperature fluorescence arises mostly from the PSII antenna. The difference in fluorescence between states 1 and 2 can be measured with a fluorescence video imaging system and used for screening mutants deficient in state transitions. States 1 and 2 can be induced by illuminating cells with light preferentially absorbed by PSI and PSII, respectively, although it is not possible to obtain cells that are entirely in state 1 or state 2 because of the partial overlap of the absorption spectra of the PSII and PSI antennae. Alternatively state 1 can be achieved by fully oxidizing the PQ pool in the presence of 3-(3,4-dichlorophenyl)1,1-dimethylurea (DCMU) which blocks electron exit from PSII. State 2 can be obtained by taking advantage of the chlororespiratory chain (Fig. 1) [36]. In the dark and under anaerobic conditions, this chain feeds electrons from the stroma into the PQ pool which becomes fully reduced and activates the LHCII kinase. Several mutants of *C. reinhardtii* deficient in state transitions were isolated using these screens through insertional mutagenesis by transforming a wild-type strain with a plasmid containing an appropriate selectable marker.

One of the first characterized mutants was *stt7*. This mutant is blocked in state 1 and fails to phosphorylate LHCII proteins under state 2 conditions [37]. Immunogold-labeling of sections of *stt7* cells with LHCII antisera revealed that the redistribution of LHCII and also of the cytochrome b_6f complex from the grana to the stroma regions of the thylakoids is strongly impaired during a transition from states 1 to 2. The nuclear gene affected by the *stt7* mutations encodes a thylakoid-associated Ser–Thr protein kinase. Although this kinase is clearly required for LHCII phosphorylation, it is not yet known whether it acts in a kinase cascade or whether LHCII is its direct substrate. Because of the presence of a potential *trans*-membrane domain in Stt7, one possible orientation is that the N-terminal end of Stt7 is in the thylakoid lumen and its catalytic kinase domain is on the stromal side where the phosphorylated residues of LHCII are located (Fig. 3). However it is also possible that the Stt7 kinase is present entirely on the stromal side of the thylakoid membrane. The large kinase domain may prevent the kinase from entering between the stacked membranes in the grana region which are separated by only 2 nm [38]. This raises the question whether LHCII phosphorylation occurs on the PSII–LHCII supercomplexes in the grana regions, at the grana–stroma boundaries or on the grana end membranes. There is less thylakoid membrane stacking in Chlamydomonas than in land plants and thus one would expect a greater accessibility of LHCII for the kinase. This may explain the larger proportion of mobile LHCII in Chlamydomonas as compared to land plants.

C. reinhardtii contains another protein kinase related to Stt7 called Stl1 which also appears to be targeted to the chloroplast based on the presence of an N-terminal chloroplast presequence. However, the function of this kinase is not yet known. These two proteins have orthologs in *A. thaliana* called STN7 and STN8, respectively (Fig. 3) [37]. The Stt7 kinase is also conserved in other plants such as rice and in marine algae. Because of the availability of Arabidopsis T-DNA insertion lines with disruptions in the *STN7* or *STN8* genes, it was possible to elucidate the function of these proteins. In this way it was shown that STN7 is required for state transitions and for the phosphorylation of several LHCII proteins under state2 conditions [39]. In contrast the phosphorylation of other major thylakoid proteins CP43, D1 and D2 still occurs in the *stn7* mutant thus indicating the specificity of the STN7 protein kinase. Furthermore, site directed mutagenesis of a Lys residue essential for the kinase activity blocks transition from states 1 to 2 indicating that the catalytic activity of Stt7/STN7 is indeed necessary for state transitions [39]. This had been assumed but never proven before. Thus Stt7 and STN7 are not only structurally but also functionally related.

The target sites on the LHCII proteins and thus the catalytic domain of the LHCII kinase are on the stromal side of the thylakoid membrane whereas the Qo site of the cytochrome b_6f complex, which is critical for the activation of the kinase, is on the lumen side. Hence the signal for kinase activation needs to be transduced across the membrane. The 3D crystal structure of the mitochondrial *bc1* complex revealed that the position of the Rieske protein, an important subunit of the

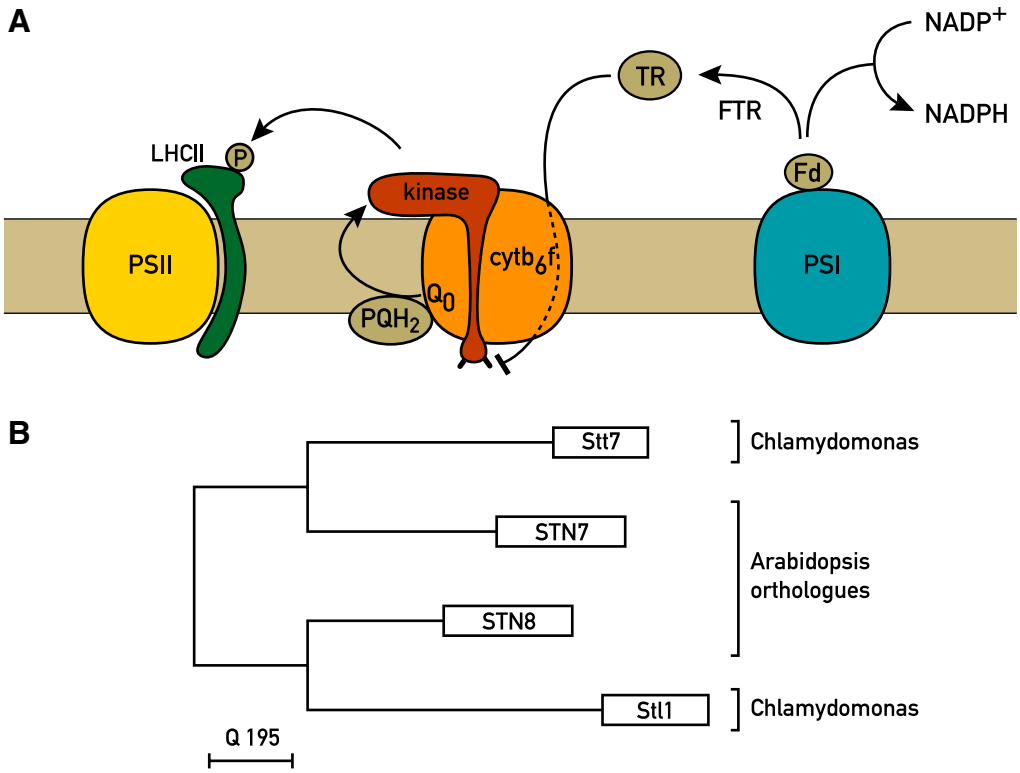

Fig. 3. Model for STN7 in the thylakoid membrane. A. The Stt7 kinase is represented with a *trans*-membrane domain with two Cys residues essential for kinase activity shown as rods exposed to the lumen. Redox control through the ferredoxin–thioredoxin system would have to be mediated through the membrane. However, it is also possible that the kinase is entirely localized on the stromal side of the membrane. Activation of the kinase upon binding of plastoquinol to the Qo site could be mediated through conformational changes in the cytochrome b_6f complex. Fd, ferredoxin, FTR, ferredoxin–thioredoxin reductase, TR, thioredoxin. B. Phylogeny of the Stt7/STN7 and Stl1/STN8 protein kinases.

complex, changes from its distal position when the Qo site is unoccupied to its proximal position when the Qo site is occupied by stigmatellin, a Qo site inhibitor [40]. This movement appears to be required for transferring electrons from the membrane soluble quinol (proximal site) to the extra-membrane heme of cytochrome *cI* (distal site). A similar movement of the Rieske protein has also been observed for the chloroplast cytochrome b_6f complex [41]. Besides the Rieske protein another candidate in this signal transduction could be subunit V, called PetO, from the cytochrome b_6f complex of *C. reinhardtii*. Amongst all the subunits of this complex, it is the only one to undergo reversible phosphorylation during state transitions [42]. One possibility is that the lumenal domain of PetO senses the structural changes of the Rieske protein and transmits them through its *trans*-membrane region to its stromal domain which could interact with the STN7 kinase.

By binding tightly to the Qo site, stigmatellin locks the Rieske protein in its proximal position, and prevents transition from states 1 to 2 and LHCII phosphorylation. However, PetO is still phosphorylated in the presence of stigmatellin [43]. In contrast PQH_2 is released rapidly from the Qo site and allows the Rieske protein to move back to its distal position. A two step process for the activation of the LHCII kinase has been proposed [43]. Upon binding of PQH_2 to Qo, the Rieske protein would move from the distal to the proximal position thereby inducing a conformational change of the cytochrome b_6f complex which would activate the kinase. This event could be marked by the phosphorylation of the PetO subunit. In the second step the return of the Rieske protein to its distal position would release the activated kinase from the cytochrome b_6f complex and allow it to interact with LHCII. This movement could be triggered by the turnover of PQH_2 at the Qo site but not by stigmatellin which blocks the Rieske protein in its proximal position. Such a dynamic model is compatible with the low abundance of the LHCII kinase compared to that of the cytochrome b_6f complex [44]. The active kinase would need to phosphorylate several PetO and LHCII substrates before it returns to its inactive state.

Another interesting feature of Stt7 and STN7 is the presence of two conserved Cys residues near the N-terminal end. A redox control of the LHCII kinase mediated through the ferredoxin/thioredoxin system has been postulated based on the findings that at high light intensities the kinase is inactivated [45]. These two Cys might be the targets of thioredoxin. If the Stt7/STN7 kinase contains a *trans*-membrane domain as predicted by one of the models (see above), one would have to postulate thylakoid *trans*-membrane redox signaling. There is a precedent for this type of signaling across the thylakoid membrane [46]. Site-directed mutagenesis of either of the conserved Cys residues in both Stt7 and STN7 leads to the loss of state transitions and LHCII phosphorylation indicating that these residues play an important role for the activation of the kinase (A. Willig, S. Lemeille and J.D. Rochaix, unpublished results). Although it is clear that the Stt7/STN7 kinase is required for LHCII phosphorylation and for state transitions, it remains to be seen whether LHCII is the direct sub-

strate of these kinases. In particular the existence of an additional kinase acting downstream of Stt7/STN7 cannot be excluded. Modification of the ATP binding sites of this kinase to allow it to accept bulky ATP analogs might be one way to test whether LHCII is the direct target of Stt7/STN7. This approach developed by Shokat has been successful in several cases [47].

The availability of Arabidopsis lines deficient in *stt7* and state transitions has made it possible to assess the role and importance of state transitions. Mutant plants grown under standard laboratory conditions are undistinguishable from wild-type plants. However, when plants were grown under changing light conditions with alternating 1 h light phases favoring PSI or PSII, the growth of *stn7* plants was significantly impaired compared to wild-type plants [39]. These plants were also tested under field conditions using an assay for plant fitness based on seed production [48]. Seed production of Arabidopsis wild-type and mutant plants impaired in the regulation of the photosynthetic light reactions grown under natural conditions in the field was compared [49]. Seed sets of plants deficient in state transitions (*stn7*) were 19% smaller than those of wild-type plants whereas plants missing the STN8 kinase, required for the phosphorylation of the core PSII reaction centre polypeptides (*stn8*), had a normal seed production. Plants lacking both the STN7 and STN8 kinases were strongly affected, indicating that these mutations act synergistically. Mutant plants (*npq4*) lacking feedback de-excitation because of the loss of PsbS [50] were severely affected in seed production. The same seed set was found in *npq4;stn7* double mutants. Based on these effects on plant fitness there appears to be a hierarchy in the importance of photosynthetic acclimation responses with, in decreasing order, feedback de-excitation, state transitions and phosphorylation of the PSII core proteins.

5. Additional roles of the Stt7/STN7 kinase

Besides inducing short term acclimation processes such as state transitions, changes in light conditions are known to lead to changes in the amounts of the antenna proteins of PSII and PSI and to a readjustment of photosystem stoichiometry in the long term [2]. This process is achieved through a signaling network involving coordinate gene expression in the nucleus and chloroplast (reviewed in [51]). The question arises whether the Stt7/STN7 kinase might also be involved in long term adaptation. When wild-type plants are illuminated for several days by light preferentially absorbed by PSI relative to PSII, the excitation pressure of PSII, as measured by the chlorophyll fluorescence parameter F_S/F_M, increases as a result of its increased antenna size and the chl *a*/*b* ratio decreases because the PSII antenna is enriched in chl *b*. The opposite occurs when the plants are illuminated with PSI light. This long term acclimation still occurs in the *stn8* mutant but not in the *stn7* mutant [52]. Under all light conditions tested the response of *stn7* is typical of plants acclimated to PSI light. In contrast to Arabidopsis we could not detect any long term acclimation response in wild-type Chlamydomonas cells subjected to a similar light regime (C. Delessert and J.D. Rochaix, unpublished results). These results raise the possibility that STN7 may also be involved in a signaling pathway that couples changes in photosynthetic performance in the short term to changes in

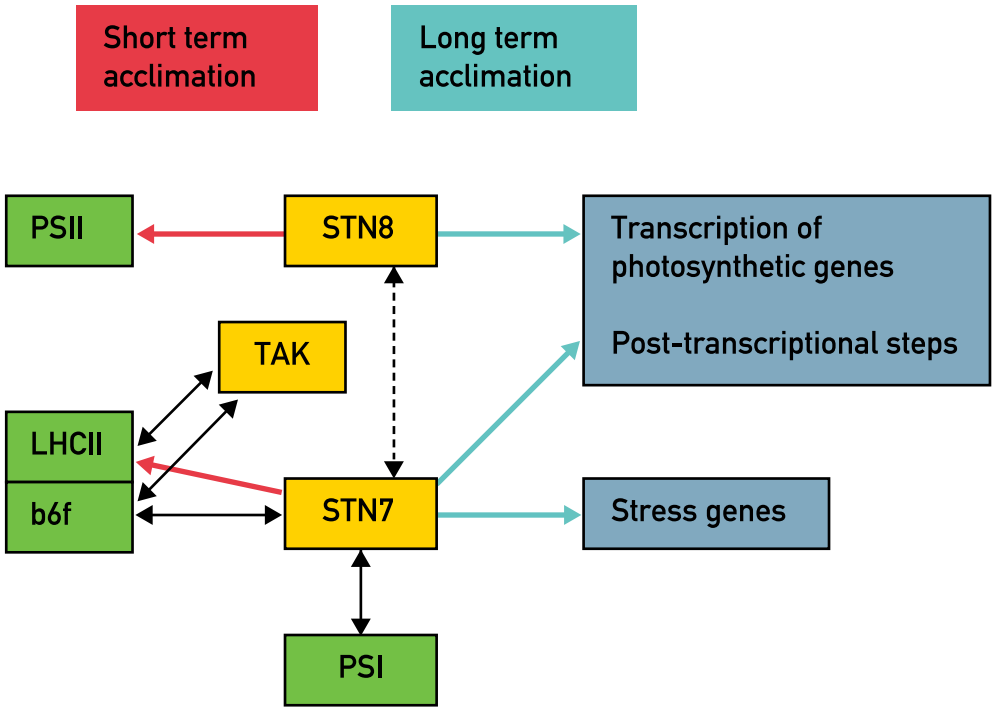

Fig. 4. Model for the multiple roles of the STN7 and STN8 protein kinases in short term and long term acclimation and in the control of expression of genes involved in photosynthesis [34,36,49–51]. The corresponding signaling chains are still largely unknown. STN7 and STN8 are required for LHCII and PSII phosphorylation, respectively (red arrows). The TAK kinase is also shown [29,30]. Double arrows indicate interactions based on co-immunoprecipitations. The genetic interaction between STN7 and STN8 is represented by a dotted line [49].

photosynthetic gene expression in the long term (Fig. 4). However, analysis of transcription patterns by microarrays did not reveal significant differences between wild type and the *stn7* mutant [52,53]. In contrast *stn8* mutant plants were strongly affected in the expression of numerous nuclear genes involved in photosynthesis. Interestingly the effect of the lack of STN8 was masked in the *stn7–stn8* double mutant in which wild-type expression patterns were restored thus indicating a genetic interaction between STN7 and STN8 [52]. At this time it is not clear how these two kinases interact with each other in the signaling network of nuclear and chloroplast gene expression.

Loss of STN7 does not appreciably affect the levels of transcripts of photosynthetic genes relative to the wild type upon long term low light acclimation. However changes in thylakoid protein content occur, notably a decrease in Lhcb1 and an increase in Lhca1 and Lhca2 as well in other chloroplast proteins of *stn7* [53]. Moreover, increases in light intensity for short periods during growth under low light conditions led to a higher increase in the expression of stress responsive genes in *stn7* than in the wild type. It has therefore been proposed that STN7 and state transitions are part of a buffering system which allows the organism to avoid over-reduction of the electron acceptors of PSI which would otherwise generate reactive oxygen species and induce the expression of stress response genes, in particular the induction of heat shock protein genes [53]. A challenge for the future will be to identify other components of the signaling network underlying short and long term light acclimation processes to changing light conditions and to determine how they act mechanistically. In particular the interactions between Stt7/STN7 and Stl1/STN8 kinases and their targets remain to be elucidated (Fig. 4).

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References

[1] Holt, N.E., Fleming, G.R. and Niyogi, K.K. (2004) Toward an understanding of the mechanism of non-photochemical quenching in green plants. Biochemistry 43, 8281–8289.
[2] Chow, W.S., Melis, A. and Anderson, J.M. (1990) Adjustments of photosystem stoichiometry in chloroplasts improve the quantum efficiency of photosynthesis. Proc. Natl. Acad. Sci. USA 87, 7502–7506.
[3] Allen, J.F. (1992) Protein phosphorylation in regulation of photosynthesis. Biochim. Biophys. Acta 1098, 275–335.
[4] Wollman, F.A. (2001) State transitions reveal the dynamics and flexibility of the photosynthetic apparatus. EMBO J. 20, 3623–3630.
[5] Bonaventura, C. and Myers, J. (1969) Fluorescence and oxygen evolution from Chlorella pyrenoidosa. Biochim. Biophys. Acta 189, 366–383.
[6] Murata, N. (1969) Control of excitation transfer in photosynthesis. I. Light-induced change of chlorophyll a fluorescence in Porphyridium cruentum. Biochim. Biophys. Acta 172, 242–251.
[7] Vener, A.V., van Kan, P.J., Rich, P.R., Ohad, I.I. and Andersson, B. (1997) Plastoquinol at the quinol oxidation site of reduced cytochrome *bf* mediates signal transduction between light and protein phosphorylation: thylakoid protein kinase deactivation by a single-turnover flash. Proc. Natl. Acad. Sci. USA 94, 1585–1590.
[8] Zito, F., Finazzi, G., Delosme, R., Nitschke, W., Picot, D. and Wollman, F.A. (1999) The Qo site of cytochrome b_6f complexes controls the activation of the LHCII kinase. EMBO J. 18, 2961–2969.
[9] Andersson, B. and Andersson, J. (1980) Lateral heterogeneity in the distribution of chlorophyll–protein complexes of the thylakoid membranes of spinach chloroplasts. Biochem Biophys. Acta 593, 427–440.
[10] Albertsson, P. (2001) A quantitative model of the domain structure of the photosynthetic membrane. Trends Plant Sci. 6, 349–358.
[11] Allen, J.F. and Forsberg, J. (2001) Molecular recognition in thylakoid structure and function. Trends Plant Sci. 6, 317–326.
[12] Bulté, L., Gans, P., Rebeille, F. and Wollman, F.A. (1990) ATP control on state transitions in Chlamydomonas. Biochim. Biophys. Acta 1020, 72–80.
[13] Finazzi, G., Rappaport, F., Furia, A., Fleischmann, M., Rochaix, J.D., Zito, F. and Forti, G. (2002) Involvement of state transitions in the switch between linear and cyclic electron flow in *Chlamydomonas reinhardtii*. EMBO Rep. 3, 280–285.
[14] Delosme, R., Olive, J. and Wollman, F.A. (1996) Changes in light energy distribution upon state transitions: an in vivo photoacoustic study of the wild type and photosynthesis mutants from *Chlamydomonas reinhardtii*. Biochim. Biophys. Acta 1273, 150–158.
[15] Elrad, D. and Grossman, A.R. (2004) A genome's-eye view of the light-harvesting polypeptides of *Chlamydomonas reinhardtii*. Curr. Genet. 45, 61–75.
[16] Minagawa, J. and Takahashi, Y. (2004) Structure, function and assembly of photosystem II and its light-harvesting proteins. Photosynth. Res. 82, 241–263.
[17] Kuhlbrandt, W., Wang, D.N. and Fujiyoshi, Y. (1994) Atomic model of plant light-harvesting complex by electron crystallography. Nature 367, 614–621.
[18] Liu, Z., Yan, H., Wang, K., Kuang, T., Zhang, J., Gui, L., An, X. and Chang, W. (2004) Crystal structure of spinach major light-harvesting complex at 2.72 A resolution. Nature 428, 287–292.
[19] Bennett, J. (1977) Phosphorylation of chloroplast membrane proteins. Nature 269, 344–346.
[20] Allen, J.F., Bennett, J., Steinback, K.E. and Arntzen, C.J. (1981) Chloroplast protein phosphorylation couples plastoquinone redox state to distribution of excitation energy between photosystems. Nature 291, 25–29.
[21] Turkina, M.V. and Vener, A.V. (2006) Identification of phosphorylated proteins (Thiellement, H., Zivy, M., Damerval, C. and Méchin, V., Eds.), Plant Proteomics: Methods and Protocols, vol. 335, Humana Press Inc., Totowa, NJ.
[22] Vallon, O., Bulte, L., Dainese, P., Olive, J., Bassi, R. and Wollman, F.A. (1991) Lateral redistribution of cytochrome *b6/f* complexes along thylakoid membranes upon state transitions. Proc. Natl. Acad. Sci. USA 88, 8262–8266.
[23] Zhang, S. and Scheller, H.V. (2004) Light-harvesting complex II binds to several small subunits of photosystem I. J. Biol. Chem. 279, 3180–3187.
[24] Lunde, C., Jensen, P.E., Haldrup, A., Knoetzel, J. and Scheller, H.V. (2000) The PSI-H subunit of photosystem I is essential for state transitions in plant photosynthesis. Nature 408, 613–615.
[25] Ben-Shem, A., Frolow, F. and Nelson, N. (2003) Crystal structure of plant photosystem I. Nature 426, 630–635.
[26] Kargul, J., Turkina, M.V., Nield, J., Benson, S., Vener, A.V. and Barber, J. (2005) Light-harvesting complex II protein CP29 binds to photosystem I of *Chlamydomonas reinhardtii* under state 2 conditions. FEBS J. 272, 4797–4806.
[27] Takahashi, H., Iwai, M., Takahashi, Y. and Minagawa, J. (2006) Identification of the mobile light-harvesting complex II polypeptides for state transitions in *Chlamydomonas reinhardtii*. Proc. Natl. Acad. Sci. USA 103, 477–482.
[28] Elrad, D., Niyogi, K.K. and Grossman, A.R. (2002) A major light-harvesting polypeptide of photosystem II functions in thermal dissipation. Plant Cell 14, 1801–1816.
[29] Pascal, A.A. et al. (2005) Molecular basis of photoprotection and control of photosynthetic light-harvesting. Nature 436, 134–137.
[30] Coughlan, S.J. and Hind, G. (1986) Purification and characterization of a membrane-bound protein kinase from spinach thylakoids. J. Biol. Chem. 261, 11378–11385.
[31] Sokolenko, A., Fulgosi, H., Gal, A., Altschmied, L., Ohad, I. and Herrmann, R.G. (1995) The 64 kDa polypeptide of spinach may

not be the LHCII kinase, but a lumen-located polyphenol oxidase. FEBS Lett. 371, 176–180.
[32] Snyders, S. and Kohorn, B.D. (1999) TAKs, thylakoid membrane protein kinases associated with energy transduction. J. Biol. Chem. 274, 9137–9140.
[33] Snyders, S. and Kohorn, B.D. (2001) Disruption of thylakoid-associated kinase 1 leads to alteration of light harvesting in Arabidopsis. J. Biol. Chem. 276, 32169–32176.
[34] Fleischmann, M.M., Ravanel, S., Delosme, R., Olive, J., Zito, F., Wollman, F.A. and Rochaix, J.D. (1999) Isolation and characterization of photoautotrophic mutants of *Chlamydomonas reinhardtii* deficient in state transition. J. Biol. Chem. 274, 30987–30994.
[35] Kruse, O., Nixon, P.J., Schmid, G.H. and Mullineaux, C.W. (1999) Isolation of state transition mutants of *Chlamydomonas reinhardtii* by fluorescence video imaging. Photosynth. Res. 61, 43–51.
[36] Bennoun, P. (1982) Evidence for a respiratory chain in the chloroplast. Proc. Natl. Acad. Sci. USA 79, 4352–4356.
[37] Depège, N., Bellafiore, S. and Rochaix, J.D. (2002) Role of chloroplast protein kinase Stt7 in LHCII phosphorylation and state transition in Chlamydomonas. Science 299, 1572–1575.
[38] Dekker, J.P. and Boekema, E.J. (2005) Supramolecular organization of thylakoid membrane proteins in green plants. Biochim. Biophys. Acta 1706, 12–39.
[39] Bellafiore, S., Barneche, F., Peltier, G. and Rochaix, J.D. (2005) State transitions and light adaptation require chloroplast thylakoid protein kinase STN7. Nature 433, 892–895.
[40] Zhang, Z. et al. (1998) Electron transfer by domain movement in cytochrome *bc*1. Nature 392, 677–684.
[41] Breyton, C. (2000) Conformational changes in the cytochrome b_6f complex induced by inhibitor binding. J. Biol. Chem. 275, 13195–13201.
[42] Hamel, P., Olive, J., Pierre, Y., Wollman, F.A. and de Vitry, C. (2000) A new subunit of cytochrome b_6f complex undergoes reversible phosphorylation upon state transition. J. Biol. Chem. 275, 17072–17079.
[43] Finazzi, G., Zito, F., Barbagallo, R.P. and Wollman, F.A. (2001) Contrasted effects of inhibitors of cytochrome b_6f complex on state transitions in *Chlamydomonas reinhardtii*: the role of Qo site occupancy in LHCII kinase activation. J. Biol. Chem. 276, 9770–9774.
[44] Gal, A., Zer, H. and Ohad, I. (1997) Redox-controlled thylakoid protein phosphorylation. News and views. Plant Physiol. 100, 869–885.
[45] Rintamaki, E., Martinsuo, P., Pursiheimo, S. and Aro, E.M. (2000) Cooperative regulation of light-harvesting complex II phosphorylation via the plastoquinol and ferredoxin–thioredoxin system in chloroplasts. Proc. Natl. Acad. Sci. USA 97, 11644–11649.
[46] Page, M.L. et al. (2004) A homolog of prokaryotic thiol disulfide transporter CcdA is required for the assembly of the cytochrome b_6f complex in Arabidopsis chloroplasts. J. Biol. Chem. 279, 32474–32482.
[47] Shah, K., Liu, Y., Deirmengian, C. and Shokat, K.M. (1997) Engineering unnatural nucleotide specificity for Rous sarcoma virus tyrosine kinase to uniquely label its direct substrates. Proc. Natl. Acad. Sci. USA 94, 3565–3570.
[48] Kulheim, C., Agren, J. and Jansson, S. (2002) Rapid regulation of light harvesting and plant fitness in the field. Science 297, 91–93.
[49] Frenkel, M., Bellafiore, S., Rochaix, J.D. and Jansson, S. (2007) Hierarchy amongst photosynthetic acclimation responses for plant fitness. Physiol. Plantarum 129, 455–459.
[50] Li, X.P., Bjorkman, O., Shih, C., Grossman, A.R., Rosenquist, M., Jansson, S. and Niyogi, K.K. (2000) A pigment-binding protein essential for regulation of photosynthetic light harvesting. Nature 403, 391–395.
[51] Pfannschmidt, T. (2003) Chloroplast redox signals: how photosynthesis controls its own genes. Trends Plant Sci. 8, 33–41.
[52] Bonardi, V., Pesaresi, P., Becker, T., Schleiff, E., Wagner, R., Pfannschmidt, T., Jahns, P. and Leister, D. (2005) Photosystem II core phosphorylation and photosynthetic acclimation require two different protein kinases. Nature 437, 1179–1182.
[53] Tikkanen, M. et al. (2006) State transitions revisited – a buffering system for dynamic low light acclimation of Arabidopsis. Plant Mol. Biol. 62, 779–793.